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Influence of Altitudes on Activity of Soil Health Bioindicators β-glucosidase and Urease in Agricultural Soils of Almora District of Central Himalaya

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ABSTRACT

The present study was aimed to study the influence of altitudes on activity of soil health bioindicators in agricultural soils of Almora district of Central Himalaya. Seventy two soil samples were collected from agricultural lands at different altitudes of Almora, Uttarakhand, India and were assayed for their physico-chemical and enzymatic activities such as β-glucosidase and urease following the standard procedures. Physico-chemical and biological properties of soil were: pH 5.2-6.78; organic C 1.05-2.44%; available N 166.84-323.4 kg ha^{-1}; available K 179.93-310.59 kg ha^{-1}; available P 21.1-31.1 kg $ha^{-1}$and microbial biomass carbon 194.89-367.76 mg kg^{-1} C. Substrate induced β-glucosidase activity ranged between 419.73-532.01 ppm *para*-nitrophenol g^{-1} soil h^{-1} while urease activity ranged from 216.99 to 395.28 ppm urea hydrolysed g^{-1} soil h^{-1}. β-glucosidase activity was positively correlated with available nitrogen (r = 0.70*), available phosphorous (r = 0.75*) and soil microbial biomass C (r = 0.88**) while urease activity was positively correlated with available phosphorous (r = 0.84**). Enzymatic activity was high in agricultural soils at high altitude. It was concluded that activity of β-glucosidase in soil might be an effective tool to assess the soil health.

Key words: Urease, β-glucosidase, altitude, soil health bioindicator, microbial activity

INTRODUCTION

Activity of diverse group of microbial population contribute pedogenic processes of soil development by influencing the organic matter decomposition and synthesis of humus which influence physico-chemical and biological properties and create a complimentary medium for biological reactions and life support in the soil environment. A major part of the Central Himalayan is covered by the forest which supports millions of resident in the region. Agriculture is being the principal activity that depends on the natural forest vegetation cover for its sustainability. Soil micro-organisms signify decomposition of the organic matter. Information on soil microbial activity of sub temperate hill ecosystems, especially at different altitudes of Central Himalaya is scanty. Soil vary in organic matter composition and hence being an important factor which influences vegetation and soil biological activity (Meyer *et al.*, 2007). It had been reported by Waring and Schlesinger (1985) that microorganisms can mineralize up to 80% of dead plant materials. Therefore, evaluation of microbial activity and related enzymes responsible for nutrient cycling is prerequisite to understand soil biological activity and fertility status of the area.

Among the various tools and techniques, enzyme activity analysis has been employed by Clegg *et al.* (2006), Bastida *et al.* (2007), Udawatta *et al.* (2009), Lodhi *et al.* (2000) and Taylor *et al.* (2002). Soil enzymes exhibit potential for microbial activity and can affect the rate of nutrient return (Sinsabaugh, 1994; Sajjad *et al.*, 2002). They can serve as an index for spatio-temporal changes in the environmental conditions because of their rapid response, even to small changes, before such changes are expressed in the organic matter content (Prietzel, 2001) and in the size of microbial biomass (Caldwell *et al.*, 1999). Soil enzyme activity is associated with living microorganisms which possess intracellular as well as extracellular activities (Li and Sarah, 2003). Therefore, enzyme activities are frequently utilized as biological indicators for soil quality and as a general indicator of microbial activity (Killham and Staddon, 2002). These biochemical processes affect the soil biological and fertility level in the long run which help the farmers (Wyszkowska and Kucharski, 2003).

In the present study extracellular enzymes in soils, β-glucosidase (EC 3.2.1.21) and urease (EC 3.5.1.5) as former being involved in the enzymatic degradation of cellulose, the main component of plant polysaccharides while later being involved in metabolism of nitrogen were assessed as bioindicators of soil health with altitudes of agricultural soil of Almora district of Central Himalaya.

MATERIALS AND METHODS

Site description and soil sampling: Soil samples were collected from Almora district of Uttarakhand, India (79°50.33" to 81°10" E longitude and 29°29.22" to 29°35.18" N latitude) which has an area reported under utilization of about 4,64,942 hectares with forest cover of 50.8% and net sown area 28.5% in 2007-08 (http://www.indiaagristat.com/default.aspx). The altitude of the study site varies from 3000 to 8450 feet above sea level with a dry period normally spanning from March to June, a rainy season from July to September and a winter season from October to February. The average temperature is 24°C, varying with the altitude and the natural vegetation is largely sal (*Shorea robusta* Gaertn.), pine (*Pinus roxburghii* Sarg.), mixed oak (*Quercus leucotrichophora* A. Camus), broadleaved oak and deodar (*Cedrus deodara* Loud.). The area is well populated having a population of 630567 (Datanet India Pvt. Ltd., Delhi) with agriculture as major activity dispersed along the slope. The principal crops are wheat, rice, barley, manduwa, gahat, rajma, black soyabean. The agriculture of the area depends mainly on rain for irrigation of the field. But in some area there is availability of the canals and seeping water. The soils belong to Inceptisols, Entisols, Mollisols and Alfisols in different regions (Singh and Singh, 1992).

A total of seventy two soil samples were collected during October 2010 from sites around Almora, Uttarakhand, India before tilling the soil (Fig. 1). The samples were collected following the standard soil sampling procedure and then transported to the laboratory at Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi in isothermic bags. Soil samples were divided in two parts. One part of the samples were air dried and processed for chemical analysis; another part of the soil samples were stored at 4°C for biological analysis.

Fig. 1: Almora district of Uttaranchal, India. ★ Sampling site

Chemical analysis: Soil samples were analyzed for pH in a soil solution of ratio 1:2.5 wt:vol, with a glass electrode (Jackson, 1973); oxidizable SOC (Walkley and Black, 1934); Kjeldahl N by Semi-Autoanalyser (Model 2200), available P following the Olsen *et al.* (1954) method and available K by 1 N NH_4OAc using a flame photometer (Jackson, 1973).

Enzymatic assay: Extracellular enzyme, β-glucosidase and urease activities were assessed in soil. β-Glucosidase (EC 3.2.1.21) was assayed by following the method as described by Hayano (1973) using the substrate analogue para-nitrophenyl-β-d-glucopyranoside (pNPG) and urease (EC 3.5.1.5) by the method of Tabatabai (1982).

A generalization regarding the action of these soil enzymes for clear under standing has been given in Table 1.

Statistical analysis: Values of a particular characteristic of soil obtained from analysis of soil samples of different locations at same altitudes were pooled together. Analyses were made in three

Table 1: Generalization about the soil enzymes under study

Class/EC No.	Recommended name	Role in soil function	Reaction catalysed	Substrate	Optimum pH
3.2.1.21	β-Glucosidase	Cellulose degradation, producing glucose needed by plants and microorganisms	Glucoside-R + H_2O →Glucose+R-OH	p-Nitrophenyl-β-D glucopyranoside (10 mM)	6.0
3.5.1.5	Urease	Urea degradation producing available form of nitrogen	Hydrolysis of urea into ammonium and carbon dioxide	Urea	7.4

replications. Data were analyzed statistically to draw conclusion of significance by using the method as described by Panse and Sukhatme (1967). The test of significance was carried out at 5 % level of significance by referring to 'F' table values.

RESULTS

The mean values for the parameters of the agricultural soils under study at different altitudes are presented in Table 2. In this study, the mean value for the pH, percent organic C, content of mineralizable N, available phosphorous and available K in soil ranged from 5.3-6.2, 1.05 to 2.44, 166.84 to 323.4 kg ha^{-1}, 21.1 to 31.1 kg ha^{-1} and 153.30 to 310.59 kg ha^{-1}, respectively. Also, the mean microbial biomass carbon for the soil sample ranged from 194.89 to 367.76 mg kg^{-1} soil.

Data on pH revealed that soil of the Almora is acidic in nature. Lowest pH (5.3) was recorded at altitude of 3000-3500 feet followed by 5.5 at 4500-5000 feet of altitude. Percent organic C tended same as pH of the soil. Accordingly for all other soil chemical parameters studied, the maximum value was observed at higher altitude. The difference in mean value with altitude for all the parameters was statistically significant at $p = 0.05$ except the pH.

Statistical analysis of the data using ANOVA in MS Excel revealed that organic C possess a strong positive correlations with mineralisable nitrogen (r = 0.81**) and potassium (r = 0.84**). Also organic carbon was positively correlated with phosphorous content (r = 0.65*) (Table 3). Soil pH showed positive correlation with the organic carbon (r = 0.73*) and mineralisable N (r = 0.77*). The available phosphorous content of the soil was found to possess positive correlation with Total N content (r = 0.76*).

β-glucosidase, an important soil enzyme involved in carbon cycle, varied from 419.73 to 532.01 ppm pNP g^{-1} soil h^{-1} while the urease activity involved in nitrogen assimilation, ranged from 216.99 to 395.28 ppm urea hydrolysed g^{-1} soil h^{-1} (Table 2). Soil microbial biomass carbon in the agricultural soil at different altitudes also showed variation ranging from minimum value of 194.89 mg kg^{-1} at 3000-3500 feet to maximum value of 367.76 mg kg^{-1} at an altitude of 6500-7000 feet. The rise or fall in the microbial activity was variable. **β**-glucosidase possessed significant positive correlation with urease (r = 0.71*), available P (r = 0.75*) and with SMBC (r = 0.88**). Similarly, urease activity was also found to be positively correlated with available P (r = 0.84**). Altitude significantly influenced the enzymatic activities erratically and its activities was highest at 6500-7000 feet of altitude for both the enzymatic activities. Lowest activity of **β**-glucosidase and urease was recorded at 5000-6000 feet of height.

Table 2: Effect of altitudes on soil health characteristics of Agricultural soil of Almora district

	Chemical soil health properties					Microbiological soil health properties		
Altitude (feet)	pH	Organic C (%)	Total N (kg^{-1})	P_2O_5 (kg^{-1})	K_2O (kg^{-1})	SMBC (mg kg^{-1})	Urease (ppm urea hydrolysed g^{-1} soil h^{-1})	β-Glucosidase (ppm pNP g^{-1} soil h^{-1})
3000-3500	5.32	1.24	189.31	21.1	187.56	194.89	267.87	453.54
3500-4000	5.56	1.32	221.82	25.4	179.93	285.05	325.66	486.01
4000-4500	5.67	1.05	241.28	26.8	153.30	312.37	270.35	488.10
4500-5000	5.52	1.41	213.75	25.5	241.10	228.71	341.51	448.78
5000-5500	5.66	1.27	166.84	22.6	245.60	258.43	225.01	435.42
5500-6000	6.05	1.51	244.32	21.7	217.76	222.28	216.99	419.73
6000-6500	5.96	1.49	234.18	22.4	253.40	302.59	264.60	498.03
6500-7000	6.19	2.44	323.4	31.1	310.59	367.76	395.28	532.01
CD (P=0.05)	NS	0.114	25.607	NS	26.802	35.870	31.104	35.372
SEM±	0.369	0.053	11.966	3.125	12.524	16.761	14.535	16.529

NS: Not significant

Table 3: Simple significant correlations coefficients of different soil health properties

	pH	OC	Total N	Available P	SMBC	Urease
pH						
Organic C	0.73*					
Total N	0.77*	0.81**				
Available P	--	0.65*	0.76*			
Available K	--	0.84**	--	--		
SMBC	--	--	0.73*	0.80**		
Urease	--	0.65*	--	0.84**	--	
β-Glucosidase	--	--	0.70*	0.75*	0.88**	0.71*

*Significant at p = 0.05, **Significant at p = 0.05

DISCUSSION

Altitude is one of the main topographic factors affecting the physicochemical and biological properties of the soil and hence the overall soil health. People of Uttarakhand and other hilly areas usually add waste product of plants in their fields to meet the nutrients requirements of the crops. They depend totally on the organic manures in the form of farm yard manures for their agriculture. But now a days some farmers also had started using chemical fertilizer specially, urea. Apart from it the increasing population density leads to the conversion of more forest land into agriculturable land.

The soil of the region show higher percent organic carbon as compared to the soils of plains. It is due to the addition of more organic waste as farm yard manure and leaf litter from plants in soil which plays a major role in increasing the carbon content of the soil. Also agriculturable soil at the high altitude has more carbon as compared to the soils at low altitude. This might be due to the conversion of forest in agriculturable land at high altitude while frequent agricultural practices at low altitude leads to the loss of organic matter from soil pool. This statement is in conformity to the findings of Abera and Belachew (2011). Hence, it has been observed that people at these altitude depends on more organic matters for the carbon source. As a result the addition of organic matter contributed to the soil N, P and K which caused highly significant positive correlation with content of percent organic C. It is true as the content of organic matter in the soil has lead to the increment of mineralisable nitrogen, phosphorous and potassium in the soil. A positive and significant correlation of organic carbon with nitrogen, phosphorous and potassium has been reported by other researchers (Rezende *et al*., 2004; Verma *et al*., 2010; Onweremadu, 2007).

Also the variation in the carbon content was significantly influenced by the variation in altitude. Consequently, the nitrogen, phosphorous and potassium content of the soil were also increased with altitude.

Addition of organic matter has been found to be good agricultural management to increase the content of organic carbon. The higher organic matter depicts the high carbon content. It has also been suggested by Pascual *et al*. (1997) and Uyanoz *et al*. (2005) that the higher biomass reflects the potential for soil's organic matter mineralization. As a result the nutrient, i.e., N, P and K content of the soil increases. The microbial biomass carbon was found to be influenced by the altitude. The agricultural soil at higher altitude has higher microbial biomass carbon.

Waring and Schlesinger (1985) reported that microorganisms can mineralize up to 80% of dead plant materials. With the increasing awareness of the soil microbial activities many studies have been conducted in this regard. Temperature, pH, soil water content, land use changes and nutrient availability have been identified as key variables controlling soil microbial activities. These facts are in evident to the experimental findings of Anderson and Nilsson (2001), Allison *et al*. (2008), Ye *et al*. (2009), Venkatesan and Senthurpandian (2006), Kang *et al*. (2003), Jia *et al*. (2006) and Enowashu *et al*. (2009).

Debosz *et al*. (1999) and Eivazi and Tabatabai (1988) have stated that **β**-glucosidase activity in the soils is ideal to examine the importance of physico-chemical controls on the turnover of soil organic matter. **β**-glucosidase is synthesized by soil microorganisms in response to the presence of suitable substrate. It has been found in the current study that its activity is significantly influenced by the variation in altitude. It was maximum (32.01) at high altitude of 6500-7000 feet and comparatively lower at lower altitudes. It was due to the variation in substrate availability in soil. The enzymatic activity at an altitude of 3500-4500 and 4500-6000 has little variation in their activity which is statistically insignificant. This might be due to limitation of the substrate in soil or some other factors may be involved for this variation to occur. Enzymatic activities at high altitude seem to be more prominent due to the availability of substrate or may be supported by the other environmental factors. These environment factors may be temperature or pH of the soil for its high activity. Substrates in the form of organic matter are the source of carbon and nitrogen for the microorganism. Xu *et al*. (2000), Xue *et al*. (2003), Wang *et al*. (2006) and Liu *et al*. (2007) deserve to mention that the kind and number of soil microorganisms determine, to a certain extent, the source of soil enzymes. Activity of the **β**-glucosidase reflects the status of labile organic C turnover in the soil, confirmed by a positive correlation between **β**-glucosidase activity and organic carbon in soil.

Variation in altitudes greatly influences the activity of urease. Urease activity was found to be greater at high altitude as compared to the lower altitude. This is due to the presence of high organic matter content in the soil as a result of shifting cultivation and addition of huge amount of organic matter and leaf litter from the trees. Beri *et al*. (1978) had reported that soil urease activity is largely controlled by the organic carbon status of the various soils. The positive correlations of urease activity with organic carbon ($r = 0.65$) suggested that organic matter content accounted for most of the variations in soil urease activity. This was in accordance to the finding of Tabatabai (1977) who reported that urease activity was significantly correlated with organic carbon.

CONCLUSION

With the increasing concern about the soil health, researchers have started working various indicators for soil health assessment. The present study concludes that while going for a biological indicator it is necessary to keep in mind the influence of the altitude. Soil enzyme, a bioindicator, shows a great variation in its activity with the altitude.

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